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# BIOLOGICAL ROLE OF PHOSPHATASE PTEN IN CANCER AND TISSUE INJURY HEALING

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## 1. ABSTRACT

PTEN (phosphatase and tensin homolog deleted on chromosome ten) also referred to as MMAC (mutated in multiple advanced cancers) was discovered as a tumor suppressor gene and later found to be a phospholipid phosphatase. PTEN negatively regulates Akt activation by preventing its phosphorylation. PTEN therefore inhibits the PI 3-kinase/Akt signaling pathway which is important for cell growth and survival. Overexpression or enhanced activation of PTEN can potentially impair injury healing by at least 4 mechanisms. PTEN can: 1) inhibit entry into the cell cycle by inhibiting G1 to S phase progression and arrest cell proliferation required for tissue reconstruction during injury healing; 2) increase apoptosis by blocking Akt activation leading to increased Bad and Caspase-9 activities; 3) inhibit hypoxia-induced angiogenesis required for injury healing by blocking Akt-mediated VEGF gene transcription; 4) inhibit Akt-mediated cell migration, i.e. re-epithelialization, which is also required for injury healing. The same mechanisms can also suppress cancer growth and metastases. Therefore, elucidating the role of the PTEN/PI 3-kinase/Akt pathway will likely advance our knowledge of the mechanisms controlling the processes of injury healing and cancer growth.

## 2. WHAT IS PTEN?

PTEN (phosphatase and tensin homolog deleted on chromosome ten) /MMAC (mutated in multiple advanced cancers) was first identified as a tumor suppressor product of a gene located at 10q23 by two groups of investigators in 1997 (1, 2). Mapping of homozygous deletions on human chromosome 10q23 led to the isolation of PTEN, and mutations of PTEN were subsequently detected in approximately 50% of prostate cancer cell lines, 30% of glioblastoma cell lines and xenografts, 20% of primary glioblastomas, and 5% of breast cancer cell lines and xenografts (1-23). Another group also showed that MMAC coding-region mutations are present in a number of glioma, prostate, kidney and breast carcinoma cell lines and in tumor specimens (2). The predicted PTEN product has a protein tyrosine phosphatase domain and extensive homology to tensin, a protein that interacts with actin filaments at focal adhesions. This homology suggested that PTEN may suppress tumor cell growth by antagonizing protein tyrosine kinases and may regulate tumor cell invasion and metastasis through interactions with focal adhesions (1). In 1997, another group identified the same gene while serching for new dual-specificity phosphatases and named it TEP-1 [TGF (transforming growth factor)-beta- regulated and epithelial cell-enriched phosphatase] (3). In TGF-beta-sensitive cells, TEP-1 expression is rapidly down-regulated by TGF-beta, a cytokine known to be involved in regulating cell adhesion and cell motility (3). PTEN is one of the most common targets of mutation in human cancers [glial tumor (glioblastoma multiforme/anaplastic astrocytoma), prostate, endometrial, renal and small cell lung carcinomas, melanoma, and meningioma (1, 2, 4-17)],

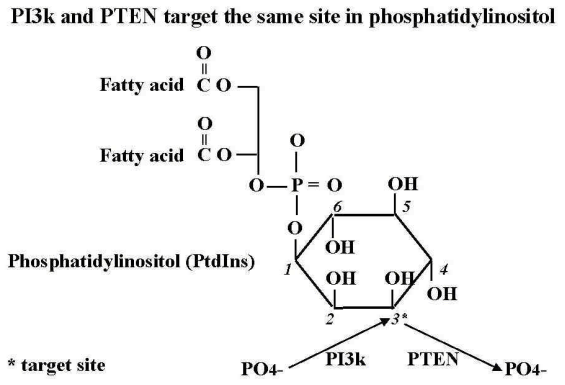


**Figure 1.** Phosphatidylinositol 3-kinase (PI3k) and PTEN target the same site in phosphatidylinositol (PtdIns). PTEN dephosphorylates the D3 position in the inositol ring of membrane phosphatidylinositols (PtdIns) that is phosphorylated by phosphatidylinositol 3-kinase (PI3k), making PI3k and PTEN a "yin and yang" enzyme pair.

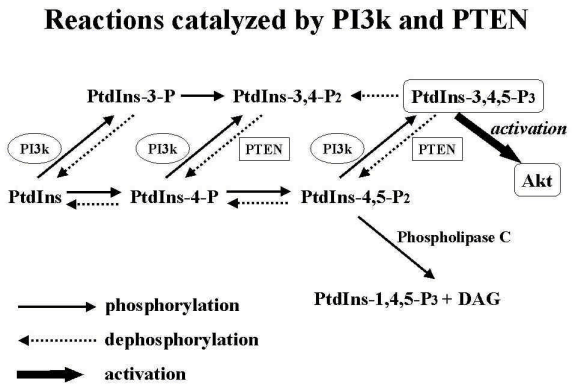


**Figure 2.** Reactions catalyzed by PI 3-kinase (PI3k) and PTEN. Phosphatidylinositol 3-kinase (PI3k) phosophorylates the D3 position of phosphatidylinositide (PtdIns), PtdIns-4-P, or PtdIns-4,5-P2 to produce PtdIns-3-P, PtdIns-3,4-P2, or PtdIns-3,4,5-P3, respectively. PtdIns-3,4-P2 can also be produced by dephosphorylating the D5 position of PtdIns-3,4,5-P3. In addition, PtdIns-3,4-P2 can be produced by phosphorylating the D4 position of PtdIns-3-P. PTEN has been shown to dephosphorylate the D3 position of both PtdIns-3,4,5-P3 and PtdIns-3,4-P2 and thus to reverse the reactions catalyzed by PI3k.

with a mutation frequency approaching that of p53. PTEN has been shown to be a phospholipid phosphatase, and it is now recognized that PTEN has the potential to regulate cellular functions important for proliferation, survival and motility (18, 19).

## 3. BIOLOGICAL ROLE AND FUNCTIONS OF PTEN

PTEN negatively regulates activation of Akt kinase by indirectly preventing its phosphorylation (18, 19). By dephosphorylating the D3 position on phosphatidylinositol (PtdIns), PTEN prevents activation of phosphoinositide-dependent kinase-1 (PDK-1) (20-25) (Figures 1, 2 & 3). Since PDK-1 activation is required for the activation of Akt, Akt activation is inhibited (20-25). Therefore, PTEN inhibits the PI 3-kinase/Akt signaling pathway essential for cell growth and survival (20-22). Cells expressing constitutively active Akt are refractory to PTEN overexpression; however, cells expressing constitutively active PI 3-kinase but wild type Akt are susceptible to the effects of PTEN overexpression (20-25). These findings indicate that PTEN inhibits Akt activity, but not PI 3-kinase activity (Figure 2). Akt is known to activate cell cycle entry and to promote cell survival by inhibiting apoptosis. Akt has also recently been shown to be involved in hypoxia-induced gene activation (e.g. VEGF) through stabilization/activation of HIF-1alpha (26, 27). In glioblastoma cell lines, PTEN negatively regulates hypoxia-induced angiogenic gene expression by inhibiting Akt-mediated stabilization of HIF-1alpha (26). Transfection of wild-type PTEN into glioblastoma cell lines lacking functional PTEN ablates the induction of HIF-1-regulated genes by hypoxia (26). Since hypoxia-induced gene activation (e.g. VEGF) by HIF is required for hypoxia-induced angiogenesis, PTEN overexpression would be expected to impair the angiogenic response to hypoxic conditions. Furthermore, Akt has been shown to mediate Rac1/Cdc42-dependent cellular migration (28). Overexpression of dominant-negative mutant forms of Rac1 and CDC42 reversed the enhanced cell migration phenotype of PTEN (-/-) cells suggesting that PTEN negatively controls cell motility through its lipid phosphatase activity by down regulating the activities of Rac1 and Cdc42 mediated by Akt (28). All of these processes (cell proliferation, survival, angiogenesis and migration) directly pertain to events required for wound healing, and also for cancer growth.

## 4. WHERE IS PTEN ENCODED AND WHERE IS IT LOCALIZED?

The PTEN gene encodes a 403-amino-acid peptide with a relative molecular mass of approximately 47 kilodaltons (1, 3). The PTEN gene is located on chromosome 10q23 (3). Immunofluorescence studies in HepG2 or NIH3T3 cells treated with TGF-beta showed that the PTEN protein is predominantly localized to the cytoplasm (3). Most reports have indicated that PTEN is primarily expressed in epithelial cells (4-17), including gastric, colon, breast, brain, endometrial, and prostate epithelial cells. However, it has recently been demonstrated that PTEN is also expressed in endothelial cells, including microvascular endothelial cells (29). Inhibition of endogeneous PTEN in cultured endothelial cells by adenovirus-mediated overexpression of a dominant negative PTEN mutant enhanced VEGF-mediated Akt phosphorylation, and this effect correlated with decreases in caspase-3 cleavage, caspase activity, and DNA degradation following induction of apoptosis by TNF-alpha (29). Overexpression of a dominant negative PTEN mutant also enhanced VEGF-mediated endothelial cell proliferation and migration, thus promoting angiogenesis (29). In contrast, overexpression of wild-type PTEN inhibited the anti-apoptotic, proliferative, and chemotactic effects of VEGF on endothelial cells (29).

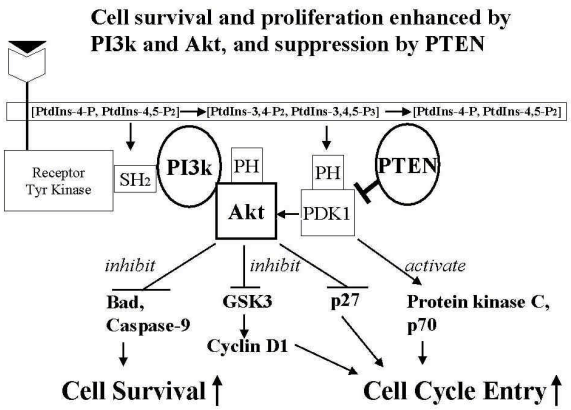


**Figure 3.** Cell survival and proliferation enhanced by PI 3-kinase (PI3k) and Akt are suppressed by PTEN. Growth factors and cytokines activate receptors that recruit PI 3-kinase (PI3k) to the plasma membrane. Phosphorylation of the membrane lipids PtdIns-4-P and PtdIns-4,5-P2 by PI 3-kinase produces the second messengers PtdIns-3,4-P2 and PtdIns-3,4,5-P3. These lipids recruit the protein-serine/threonine kinases Akt and PDK1 to the membrane and induce a conformational change in Akt, exposing its activation loop. Phosphorylation of Akt at Thr-308 of the activation loop by PDK1 turns on the protein kinase activity. Phosphorylation of Akt at a C-terminal site causes further activation. Akt phosphorylates and compromises the function of Bad and Caspase-9, proteins involved in cell death pathway. Akt also phosphorylates and inhibits glycogen synthase kinase 3 (GSK3). GSK3 phophorylates Cyclin D, targeting it for proteolysis; thus Akt may promote Cyclin D accumulation by inactivating GSK3. PDK1 also phosphorylates and enables activation of p70 and protein kinase C (PKC) family members. PTEN turns off the pathway by dephosphorylating the D3 position of PtdIns-3,4-P2 and PtdIns-3,4,5-P3.

## 5. REACTIONS CATALYZED BY PI 3-KINASE AND PTEN

Phosphoinositides (PtdIns) are phosphorylated to PtdIns-1,4,5-P3 (PIP3) and diacylglycerol (DAG) (Figure 2) by PI 3-kinase. PtdIns-3,4,5-P2 (PIP3) activates PDK-1 which, in turn, activates Akt. PTEN dephosphorylates PtdIns-3,4,5-P3 (PIP3) and, thereby, indirectly inhibits Akt activation by preventing activation of PDK-1 (22, 30-32) (Figure 3).

## 6. CELL SURVIVAL AND PROLIFERATION ARE ENHANCED BY PI 3-KINASE AND AKT, AND ARE SUPPRESSED BY PTEN

Growth factor binding to tyrosine kinase receptors triggers receptor activation and recruitment of PI 3-kinase to the plasma membrane. The activated p110 catalytic subunit of PI 3-kinase then catalyzes the phosphorylation of membrane-associated PtdIns to PtdIns-3, 4, 5-P3. The latter causes the activation of PDK-1 which, in turn, leads to activation of Akt. Akt activation promotes cell survival by de-activating Bad and Caspase-9 important for apoptosis. Activated Akt also accelerates cell cycle entry by de-activation of GSK3 (glycogen synthase kinase 3) leading to increased Cyclin D1 expression and decreased expression of p27 (Kip1), a negative regulator of cyclin dependent kinases (CDKs). PTEN indirectly blocks Akt activation and this blockage leads to increased apoptosis and cell cycle arrest (22, 23, 25, 30-32) (Figure 2).

## 7. OVEREXPRESSION OR ENHANCED ACTIVATION OF PTEN CAN POTENTIALLY IMPAIR INJURY HEALING BY AT LEAST 4 MECHANISMS AS FOLLOWS

1) PTEN inhibits cell cycle entry by inhibiting G1 to S phase progression. This is due to decreased Cyclin D activity and increased p27 expression. Since cell proliferation is essential for tissue injury healing, its inhibition by prevention of cell cycle entry would impair healing. In Cowden's disease (characterized by the occurrence of multiple hamartomas in the skin, gastrointestinal tract, breast, thyroid, and central nervous system and an increased incidence of breast and thyroid cancers), mutations in PTEN result in loss of G1 cell cycle arrest (21). Reintroduction of PTEN into PTEN-null cells restores the G1 phase block (21). Renal carcinoma cells lacking PTEN contain high levels of activated Akt, clearly indicating that PTEN is necessary for appropriate regulation of the PI 3-kinase/Akt pathway (21). In human glioblastoma U87MG cells, inhibition of the G1 to S phase transition of cell cycle progression is strongly correlated with a significant increase of the cell cycle kinase inhibitor p27 (Kip1) and a concomitant decrease in the activities of the G1 cyclin-dependent kinases (33). In embryonic stem (ES) cell lines (34) and prostate tumor cell lines (35), the accelerated G1/S transition was accompanied by down-regulation of p27 (Kip1).

2) PTEN stimulates apoptosis by increasing the activities of Bad and Caspase-9 that promote apoptosis (22-25). If apoptosis is increased to such degree that it can no longer be compensated for by cell proliferation, tissue injury healing will be impaired. Bad and Caspase-9 are pro-apoptotic proteins which are phosphorylated and inactivated by Akt. Akt was shown to directly phosphorylate pro-caspase-9, thus preventing its proteolytic activation, and the initiation of apoptosis. Thus, activation of Akt prevents apoptosis through down regulation of Bad and Caspase-9 activity while the pro-survival activity of Akt is inhibited by PTEN. The overexpression of PTEN in LaCap prostate carcinoma cells decreased cell survival (22, 23). PTEN-null embryonic stem (ES) cells showed increased levels of phosphorylated Akt and phosphorylated Bad (24).

3) PTEN can potentially inhibit hypoxia-induced angiogenesis by decreasing stabilization of the HIF transcriptional factor. HIF-1 is a heterodimer composed of HIF-1alpha and HIF-1beta subunits. The active HIF-1 complex accumulates in the nucleus and binds to the hypoxia response element (HRE) within the promoters of hypoxia-inducible genes such as VEGF, thus enhancing transcription (26, 27, 36). The availability of HIF-1 is mainly determined by the presence or absence of HIF-

**Table 1.** Overexpression of PTEN can potentially impair tissue injury healing by at least 4 mechanisms

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|---|
| 1. PTEN inhibits entry into the cell cycle.<br>PTEN negatively controls the G1 to S phase cell cycle transition by regulating the level of p27 (Kip1), a CDK inhibitor. |
| 2. PTEN increases apoptosis by blocking Akt activation.<br>Outcome: cell proliferation is overtaken by cell death. |
| 3. PTEN inhibits hypoxia-induced angiogenesis by preventing HIF-1alpha-dependent activation of VEGF.<br>Outcome: impaired induction of hypoxia-induced angiogenesis required for healing. |
| 4. PTEN inhibits Akt mediation of Rac1/Cdc42-regulated cell migration.<br>Outcome: impaired cell migration required for re-epithelialization and healing. |

**Table 2.** PTEN inhibition promotes cancer progression by 3 mechanisms

| |
|---|
| 1. Increased entry to cell cycle and thus increased proliferation due to:<br>Loss of p27<br>Increased Cyclin D1 |
| 2. Cell survival is increased due to increased Akt activity resulting in reduced apoptosis. |
| 3. Hypoxia-induced angiogenesis is increased due to:<br>Abnormally high VEGF expression from<br>Akt- induced HIF-1alpha accumulation.<br>This leads to increased tumor growth and metastasis |

1alpha (36). Akt is required for HIF-1alpha stabilization and transcriptional activity (26, 27, 35). Since HIF-1 binds to and activates the VEGF gene promoter, reduced HIF-1alpha stabilization/activity leads to (i) reduced hypoxia-induced VEGF expression and therefore (ii) reduced hypoxia-induced angiogenesis required for injury healing (37). In glioblastoma-derived cell lines, PTEN regulates hypoxia-induced angiogenic gene expression (e.g. VEGF) by regulating Akt mediated HIF-1alpha stabilization. Transfection of wild type PTEN into glioblastoma cell lines lacking functional PTEN ablates hypoxia-induced gene activation by HIF-1alpha (26). In these cells, Akt activation leads to HIF-1alpha stabilization whereas PTEN attenuates hypoxia-induced HIF-1alpha stabilization (26).

4) PTEN can potentially impair cell migration required for re-epithelialization through inhibition of Akt. Akt mediates Rac/Cdc42-regulated cell migration (28). In PTEN(-/-) mouse fibloblast cell lines, there are significant increases in the endogenous activities of Rac1 and Cdc42, two small GTPases involved in regulating the actin cytoskeleton necessary for cell motility (28). In these cells, cell migration was also enhanced. Overexpression of dominant-negative mutant forms of Rac1 and Cdc42 reversed the enhanced migration phenocyte of the PTEN(-/-) fibroblast cells (28) (Table 1).

## 8. PTEN ABROGATION IS IMPLICATED IN CANCER PROGRESSION

Based on the above description of the inhibitory activities of PTEN on cell cycle entry and proliferation, cell survival, hypoxia-induced angiogenesis and cell migration, it can be postulated that loss or reduction of PTEN may promote cancer progression by reduction or abrogation of inhibitory functions. Although not yet fully characterized, loss or mutation of PTEN is likely to play a regulatory role in cancer progression by:

1. Promoting entry into the cell cycle (proliferation) by loss of p27 (Kip 1) and increased Cyclin D1 activity resulting from constitutively activated Akt. Akt-catalyzed phosphorylation of the serin/threonin kinase, GSK3, results in GSK3 inhibition, and GSK3 promotes cyclin D proteolysis. Thus by catalyzing GSK3 inhibition, Akt contributes to cyclin D accumulation and cell cycle entry leading to increased proliferation (22).

2. Cell survival would also be increased by reduced apoptosis mediated through constitutively active Akt. The balance between cell proliferation, which would already be increased by factors such as those described in 1) above, and apoptosis would be disrupted favoring neoplastic growth (22, 23, 25, 35). For example, viral infections leading to tumor formation can be attributed in many cases to the up-regulation of anti-apoptotic (or survival) mechanisms in response to viral signaling or in response to oncogene activation (38, 39).

3. Hypoxia-induced angiogenesis would increase through abnormally high VEGF expression as a result of Akt-mediated HIF-1alpha stabilization. Tumor growth results in a hypoxic state which eventually gives rise to hypoxia-induced angiogenesis, increased tumor growth and metastasis. Stabilization and activation of HIF-1alpha by constitutively activated Akt, even under relatively mild hypoxic conditions, would be expected to further enhance tumor growth and metastasis. In addition, when tumor growth exceeds vascular density, the tumor develops a nonvascularized area in which metabolic byproducts, acidosis, low growth factor and nutrient supply, as well as hypoxia, stimulate apoptosis. Apoptosis driven by the tumor microenveronment could potentially select for loss of negative regulators of apoptosis, such as PTEN, as has been shown for other tumor suppressors that regulate apoptosis, such as p53 (26) (Table 2). Such selection would further favor the growth-promoting effects of increased Akt activation.

## 9. REGULATION OF PTEN ACTIVITY

Recently, it has been shown that the phosphorylation state of PTEN is important for the regulation of its activity (40-42).

The PTEN protein consists of three parts: (i) an amino-terminal phosphatase domain (PHD), (ii) a lipid binding C2 domain (C2D), and (iii) a 50-amino-acid C-

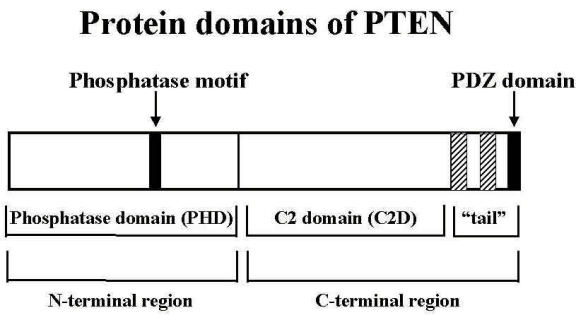


**Figure 4.** Protein domains of PTEN. PTEN is a 403-amino- acid protein which contains a tyrosine phosphatase domain (residues 1-185) in the N-terminal region with the phosphatase motif (HCXXGXXR; residues 123-130) essential for its tumor-suppressor activity. PTEN contains a C2 domain (C2D) (residues 186-351), which allows for the binding of PTEN to phospholipids, perhaps for the effective positioning of PTEN at the membrane. Proline-, glutamic acid-, serine- and threonine-rich (PEST) sequences (degradation motif) are located between residues 350-375 and 379-396 within the "tail" region (indicated by stripes). The tail region cintains casein kinase 2 (CK2) phosphorylation sites important for the stability and activity of PTEN. CK2 is a serine/threonine kinase that is ubiquitously expressed and phosphorylates a variety of substrates involved in the cell cycle and cell growth. There is also a PDZ domain which allows PTEN to bind membrane-associated guanylate kinase inverted (MAGI) proteins, which may enhance the efficiency of PTEN signaling through the formation of PTEN/MAGI complexs at the membrane. (The name PDZ derives from three proteins that contain repeats of this domain: (i) mammalian postsynaptic density protein, PSD-95; (ii) *Drosophila* disc large tumor suppressor, Dlg; and (iii) the mammalian tight junction protein, Zo-1.)

terminal "tail" that contains a PDZ binding domain (Figure 4). The tail region contains casein kinase 2 (CK2) phosphorylation sites important for the stability and activity of PTEN. CK2 is a serine/threonine kinase that is ubiquitously expressed and phosphorylates a variety of substrates involved in the cell cycle and cell growth (43). The PTEN tail is necessary for maintaining protein stability and also acts to inhibit PTEN function (41). Thus, removing the tail results in a loss of stability but also results in a protein that is more active (41). The tail-dependent regulation of stability and activity is linked to the phosphorylation of three residues (Ser380, Thr382, and Thr383) within the tail (41). These findings indicate that the PTEN tail negatively regulates PTEN function through phosphorylation.

Furthermore, phosphorylated PTEN exists in a monomeric "closed" conformation and has low affinity for PDZ domain-containing proteins [The name PDZ derives from three proteins that contain repeats of this domain: (i) mammalian postsynaptic density protein, PSD-95; *Drosophila* disc large tumor suppressor, Dlg; and the mammalian tight junction protein, Zo-1 (44).] (42). Coversely, when unphosphorylated, PTEN is in an "open" conformation and is recruited into a high molecular weight complex (PTEN-associated complex) that strongly interacts with PDZ-containing proteins such as membrane-associated guanylate kinase inverted (MAGI)-2 (43). As a consequence, when compared with wild-type PTEN, the phosphorylation-deficient mutant form of PTEN strongly cooperates with MAGI-2 to block Akt activation (42). These findings suggest that the phosphorylation of the PTEN tail suppresses the activity of PTEN by controlling the recruitment of PTEN into the PTEN-associated complex (PAC).

## 10. CONCLUSIONS

PTEN inhibits the PI 3-kinase/Akt pathway and consequently increases apoptosis and inhibits entry to the cell cycle, hypoxia induced angiogenesis and Akt regulation of Rac1/Cdc42-mediated migration required for tissue injury healing. Elucidation of the roles of the PTEN/PI 3-kinase/Akt pathway during tissue injury healing and cancer progression will advance our knowledge of the molecular mechanisms controlling these processes. New pharmacological agents that activate or suppress PTEN may find application not only for the treatment of cancer but also for tissue injury healing.

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**Abbreviations:** Akt, the cellular homolog of the viral oncogene v-akt; PTEN, phosphatase and tensin homologue on chromosome ten; PI 3-kinase, phosphatidylinositol 3-kinase; TGF-beta, transforming growth factor-beta; VEGF, vascular endothelial growth factor; PDK, phosphoinositide-dependent kinase; HIF-1alpha, hypoxia inducible factor-1alpha; GSK3, glycogen synthase kinase 3; SH, Src homology; PH, pleckstrin homology; IGF, insulin-like growth factor; DAG, diacylglycerol; CDK, cyclin dependent kinase; PHD, phosphatase domain; C2D, C2 domain; CK2, casein kinase 2; PAC, PTEN-associated complex; PDZ, postsynaptic density protein, disc-large and zo-1; PEST, proline-, glutamic acid-, serine- and threonine-rich

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